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Diversity and Rearrangement of the Human T Cell Rearranging γ Genes: Nine Germ-Line Variable Genes Belonging to Two Subgroups

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Summary

We describe nine T cell γ variable (V) gene segments isolated from human DNA. These genes, which fall into two subgroups, are mapped in two DNA regions covering 54 kb and probably represent the majority of human Vγ genes. One subgroup (Vγl) contains eight genes, consisting of four active genes and four pseudogenes. The single Vγll gene is potentially active. Sequence analysis of the Vγl genes shows variation clustered in hypervariable regions, but somatic variability is restricted to N-region diversity. Studies on rearrangement in T cell lines and in thymic DNA show that major rearrangements can be observed that are attributable to the five active Vγ genes. In addition, human cells with the phenotype of helper T cells can undergo productive Vγ–Jγ joining.

Introduction

The recognition of foreign antigen by vertebrate immune systems is mediated by B and T cells, each of which synthesize a surface antigen receptor. The T cell receptor has recently been characterized and found to be a heterodimer of α and β polypeptides (Allison et al., 1982; Haskins et al., 1983; Meuer et al., 1983). The genes responsible for synthesis of these chains have been cloned and shown to undergo rearrangement in T cells. Each locus is comprised of variable (V), joining (J), and constant (C) gene segments, which are involved in these rearrangements (reviewed by Hood et al., 1985); additional diversity (D) segments have been identified in the β locus (Clark et al., 1984; Siu et al., 1984).

The ability of T cells to recognize foreign antigen in the context of self major histocompatibility (MHC) molecules is an important facet of T cell recognition that is dissimilar to B cell recognition (Schwartz, 1985). The contribution of α and β chains in the recognition of MHC is unknown. A third gene, designated γ, has recently been discovered (Saito et al., 1984), and it has been suggested that the product of this gene may play a role in the recognition of class I MHC molecules (Heilig et al., 1985). The T cell γ locus undergoes rearrangement in mouse (Kranz et al., 1985; Hayday et al., 1985) and human DNA (LeFranc and Rabbitts, 1985; Murre et al., 1985), employing V, J, and C gene segments (Hayday et al., 1985) analogous to those of the T cell receptor α and β genes. In the mouse, three Vγ genes have been identified as well as three Cγ genes, each with a single Jγ segment (Hayday et al., 1985). However, the rearranged γ genes in mouse cytotoxic T cells (CTL) seem to be assembled from the same germ-line V and J segments (Kranz et al., 1985). Thus, variation of the γ gene in mouse CTLs is restricted to diversity created at the V–J junction (Kranz et al., 1985). In humans, on the other hand, we have observed a number of different rearranged γ gene fragments in various T cell leukemias, which represent rearrangements associated with two Cγ genes (LeFranc and Rabbitts, 1985). To examine further T cell γ diversity in man, we have cloned and mapped nine genomic Vγ genes belonging to two subgroups. In this paper we discuss patterns of rearrangement and diversity in the human T cell γ system, and we show that five major rearrangements can occur involving both Vγ subgroups. These rearrangements can involve productive joins in a number of human T cell types, including those with T helper phenotype.

Results

Germ-Line Organization of Human T Cell Vγ Genes

Unrearranged (germ-line) human Vγ genes were isolated in two stages. First, phage libraries prepared from the genomic DNA of four T cells (two cell lines SUP-T1; Smith et al., 1984) and K1010 (A. Karpas, personal communication) and two primary T cell tumors, AT5B1 (Taylor et al., 1981) and F8 (Brito-Babapulle et al., 1986) were screened with a Jγ probe, M13H60 (LeFranc and Rabbitts, 1985). Rearranged Vγ–Jγ genes were isolated in each case (the restriction maps of these clones are shown in Figure 1C and discussed in detail below) and a Vγ probe prepared from λS12, which contained a rearranged Vγ gene from SUP-T1 cells. This Vγ probe (S12SR, a 1.2 kb fragment containing the Vγ plus 300 bases of the Jγ region, see Figure 2A) was used to screen a λ phage library prepared from a B cell line (SH) to isolate unrearranged γ genes. In this way we isolated a set of unrearranged Vγ clones (Figure 1B) and rearranged clones (Figure 1C). The rearrangements found in the DNA of each of the T cells are summarized in Table 1 (these are discussed in detail below). These results allowed us to prepare a map of the germ-line organization of nine Vγ gene segments (Figure 1A), the location of which was identified by hybridization and nucleotide sequencing.

Four λ phage clones isolated from the B cell DNA library contained a total of six different unrearranged Vγ genes; λSH2,3,4, and 5 contained genes designated Vγ1, Vγ2, and Vγ3, while λSH7 contained genes designated Vγ5, Vγ6, and Vγ7. The genes Vγ4, Vγ8, and Vγ9 did not occur in any of the B cell-derived phage clones; these genes were mapped from their occurrence in clones containing rearranged genes (Figure 1C). Vγ4 was found to be rearranged in λS6 and λS13. These λ clones also contained unrearranged copies of Vγ2 and Vγ3, thereby confirming

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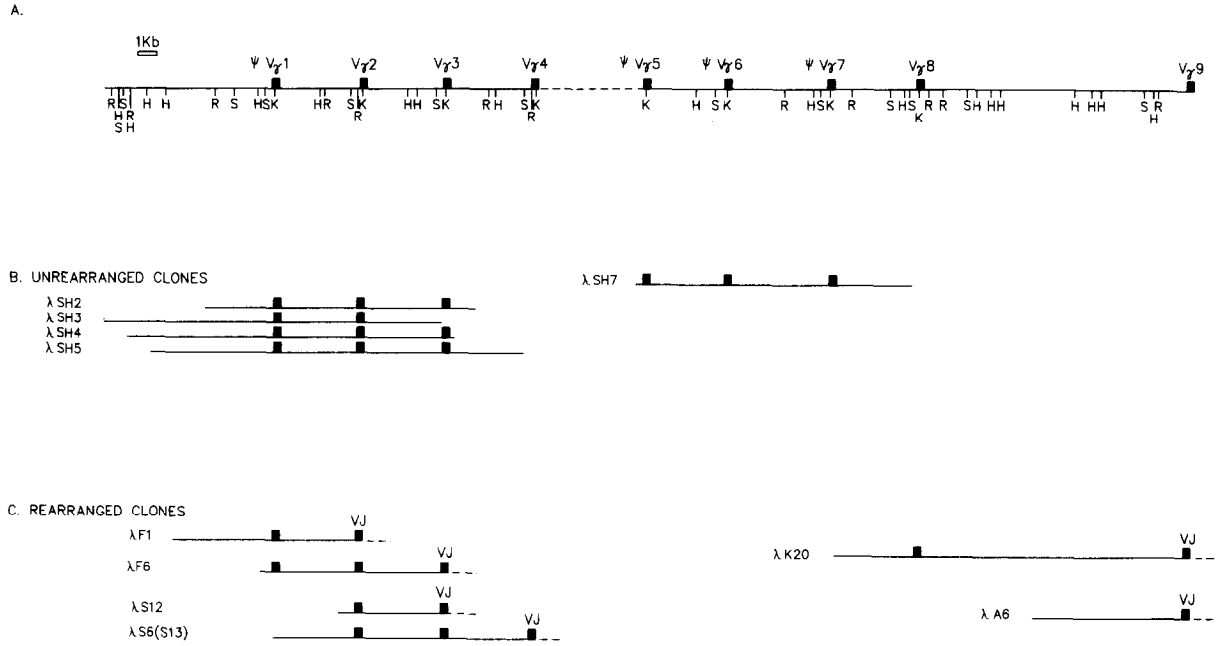


Figure 1. Map of Genomic Region Encompassing Human T Cell γ Variable Region Genes

(A) Map of germ-line Vγ genes in two sets (24 kb and 30 kb) from human DNA deduced from the organization of unrearranged (B) or rearranged (C) Vγ genes. R: EcoRI. H: HindIII. S: SacI. K: KpnI. The dotted line depicts the position between the two Vγ sets, which is of unknown length. The genes designated Vγ2, Vγ3, Vγ8, and Vγ9 have been previously described as $V\gamma^a$, $V\gamma^b$, $V\gamma^c$, and $V\gamma^d$ respectively (LeFranc et al., 1986).

(B) Lambda phage clones containing unrearranged Vγ genes isolated from a λ phage library prepared from a B-lymphoblastoid cell line (SH). The probe for screening this library was S12RS (indicated in Figure 2).

(C) Lambda phage clones containing a rearranged and linked unrearranged Vγ genes isolated (using the probe M13H60; LeFranc and Rabbitts, 1985) from the various T cell DNA libraries. λF1 and λF6 derive from F8 DNA; λS12, S6, and S13 from SUP-T1 DNA; λK20 from K1010 DNA; and λA6 from AT5B1 DNA. The rearranged Vγ gene is indicated by VJ, and the dotted line at the 3′ end of each clone indicates the start of the correspondence to the germ-line Jγ–Cγ regions.

the overlap between these Vγ segments. Two sets of phage clones, isolated from AT5B1 (named λA6) and from K1010 DNA (λK20), showed rearrangements of the gene designated Vγ9, and λK20 carried the unrearranged Vγ8 gene. The linkage of Vγ7 and Vγ8 was deduced from the region of overlap between λSH7 and λK20 (Figures 1B and 1C). The restriction maps of the ends of these clones were identical, and the overlap was confirmed by sequence analysis from the ends of the 2 kb EcoRI–SacI fragment, corresponding to the region between Vγ7 and Vγ8 (data not shown).

The data on the various λ phage clones shown in Figures 1B and 1C allowed production of a map, covering 54 kb of DNA, of two sets of human Vγ gene sequences; the first set contains four and the second set five Vγ genes (Figure 1A). Genomic hybridization was used to estimate the proportion of Vγ genes, represented in our genomic clones, using the Vγ probe S12SR (Figure 2A). This probe cross-hybridizes with all the genes from Vγ1 to Vγ8, but not with Vγ9, (LeFranc et al., 1986) and detects five main hybridizing bands in a control genomic DNA. One band (about 4.5 kb) is particularly intense and probably represents comigrating Vγ genes (Figure 2A). Thus, the Vγ probe detects a small set of genes, which probably correspond to the Vγ subgroup I in our germ-line map (Figure 1A).

The patterns of Vγ gene hybridization in genomic DNA of the T cells used here are different from those of nonlymphoid DNA and are different from each other. The T cells F8 and SUP-T1 (S in Figure 2A) show a simplified pattern compared with the control DNA. The T cells K1010 (K) and AT5B1 (A), on the other hand, have a pattern that is very similar to the control DNA, with the exception of a single rearranged band that is common to both cells. This indicates that gene rearrangement in F8 and SUP-T1 DNA had resulted in Vγ gene deletion and, therefore, that Vγ3 and Vγ4 (rearranged in F8 and SUP-T1, Table 1) are among the most distant Vγ genes from the Cγ genes. As judged by the intensity of hybridizing bands in K1010 and AT5B1 (Figure 2A), it seemed possible that no Vγ deletion had occurred in these DNAs. In this case we would expect that the region immediately upstream of the rearranged Vγ gene in AT5B1 and K1010 would be present in these two cells but lost in cells, such as F8 and SUP-T1, where upstream Vγ genes are rearranged. This was analyzed as shown in Figure 2B. A fragment from the end of λA6 (a rearranged clone isolated from AT5B1) was used in hybridizations with various genomic DNAs (Figure 2B). This probe detects an unrearranged 4 kb fragment in AT5B1, K1010, and in a control DNA (Colo320), but no hybridization was seen in F8 or SUP-T1 DNA, showing that this segment is deleted from both chromosomes in the latter two cells. Therefore, the Vγ genes rearranging in F8 and SUP-T1 would seem to be upstream of Vγ9 in the

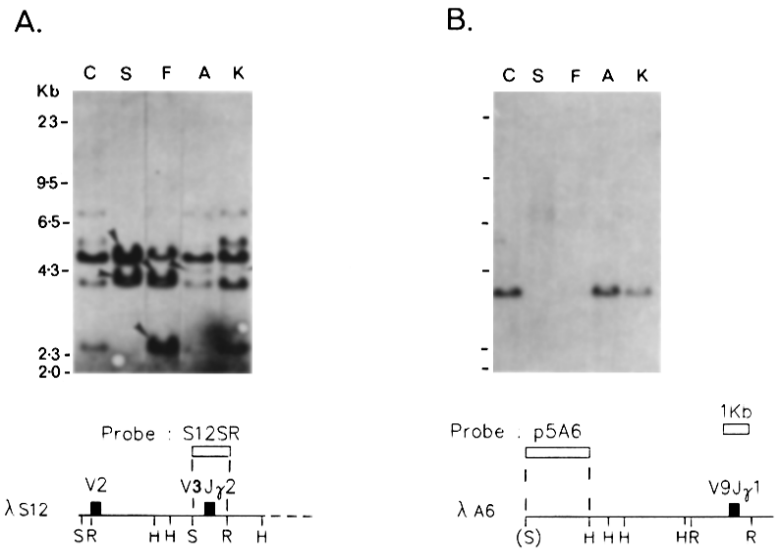


Figure 2. Orientation of the Two Vγ-Containing Genomic Regions

(A) Filter hybridization of genomic DNA, digested completely with HindIII, using the Vγ-containing probe S12RS, subcloned in pUC, from λS12 indicated at the bottom. The DNA samples applied to the gel used in this blotting experiment were Colo320 (C), SUP-T1 (S), F8 (F), AT5B1 (A), and K1010 (K). Filter was washed with 6× SSC with 0.1% SDS. Sizes were estimated by coelectrophoresis of λ DNA cut with HindIII. The bands corresponding to rearranged genes in SUP-T1, F8, AT5B1, and K1010 are marked with arrowheads. The upper rearranged band in SUP-T1 (corresponding to the Vγ4 gene) and the lower band in F8 (corresponding to the Vγ2–JγP join) comigrate with unrearranged bands.

(B) Genomic filter hybridization using HindIII-digested DNA (labeling as in A) and probed with the clone p5A6 prepared from the region of λA6 upstream of the rearranged Vγ gene (indicated at the bottom). The filter was washed with 0.1× SSC and 0.1% SDS.

Table 1. The Rearranged Vγ Genes Isolated from Various Human T Cell Sources

Cell	Type	Surface Phenotype	Rearrangement	λ Clone
F8	T-PLL	$T3^+T4^+T8^-$	Vγ3-Jγ1 Productive Vγ2-JγP Nonproductive	λF6 λF1
SUP-T1	T-lymphoma	$T3^+T4^+T8^+$	Vγ3-Jγ2 Productive Vγ4-Jγ2 Nonproductive	λS1 λS13
K1010	Transformed cord T cell	ND	Vγ9-Jγ1 Productive Unrearranged	λK20
AT5B1	T-CLL	$T3^+T4^-T8^+$	Vγ9-Jγ1 Nonproductive Unrearranged	λA6

ND: not determined.

germ-line DNA. The order of genes depicted in Figure 1A therefore seems to correspond to that of the genome, in which the Cγ genes would be to the right hand side (assuming a simple deletion mechanism of Vγ–Jγ joining).

At Least Five Active and Four Pseudo Vγ Genes in Human DNA

Nucleotide sequence analysis of the Vγ genes was undertaken to assess the variability in these genes. The nucleotide sequences of Vγ1 through to Vγ8 are compared in Figure 3 from the presumptive ATG initiation codon to the conserved heptamer/nanomer sequences that are the putative recombination signals (spaced at 23 bases from each other) at the 3′ end of the genes. Analysis of the data for the various genes shows that Vγ2,3,4,8, and Vγ9 are potentially active but Vγ1,5,6, and 7 are probably pseudogenes (discussed below). Because Vγ2 is the first potentially active gene in the set, the comparisons have been made to this gene (Figure 3). Clearly, the Vγ genes are considerably homologous to each other and constitute a V gene subgroup (VγI). Considerable nucleotide drift is apparent in the intron (nucleotides 44–168), and three other major regions of variability occur in the coding region (Figure 3). These regions correspond to hypervariable regions in the mature Vγ protein (Figure 4). We have previously shown that Vγ9 has a nucleotide sequence that differs greatly from the VγI subgroup (LeFranc et al., 1986), so this gene belongs to a separate subgroup, VγII. Genomic hybridization experiments indicate that this subgroup only has one member (unpublished observations).

Analysis of protein sequences from the Vγ genes (Figure 4) supports the idea that Vγ1 to Vγ8 belong to one subgroup, and Vγ9, to a second, distinct subgroup. Clear framework regions and hypervariable regions are apparent in the active genes. The amino acid variation within the products of active subgroup I genes is between 76% and 91% (Table 2), compared with homology of approximately 30% between the products of subgroups I and II.

Sequencing of the VγI genes revealed that each gene contains a site for the restriction enzyme KpnI. This enabled us to locate and orient the Vγ coding regions within the various λ clones and therefore to locate the genes in the genomic map represented in Figure 1A. Furthermore, from restriction mapping of the clones and the presence of the KpnI site, we could show that all of the Vγ genes have the same transcriptional orientation, that is, in the direction of Vγ1 to Vγ9.

A number of reasons compel us to consider four of the VγI subgroup genes as pseudogenes. The Vγ1 gene has a 14 bp deletion between the heptamer and nanomer se-

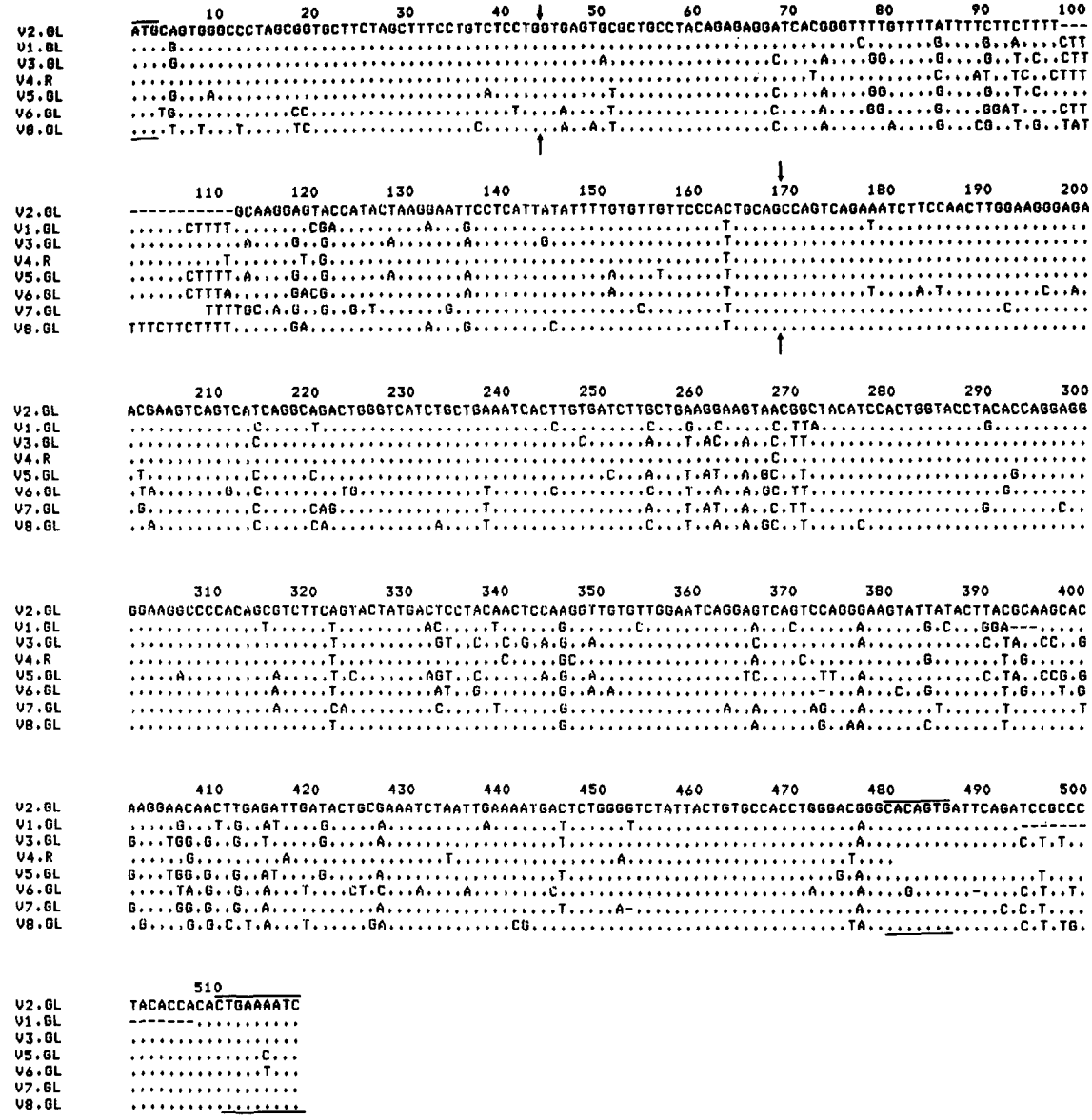


Figure 3. Nucleotide Alignment of the Human Vγl Subgroup Genes

The nucleotide sequences are indicated from the presumptive ATG codon (underlined at the start of the sequence), at the start of the leader sequence, through to the conserved heptamer and nanomer sequences at the 3′ end of the sequences (underlined at the end of the sequence). Only the full sequence of Vγ2 is given; positions of similarity in the other genes are indicated by a dot, and differences are shown by the relevant nucleotide change. The RNA splice donor (position 43) and acceptor (position 168) sites are indicated by arrows. In the left-hand column GL refers to germ-line and R refers to rearranged. The sequences were obtained from the unrearranged genes present in λSH4, λSH7, and λK20 except for Vγ4 (V4.R), which is the rearranged gene from λS6. Positions with a gap in a gene relative to Vγ2 are indicated by a dash; the pseudogenes Vγ6 and Vγ7 have such gaps in the coding region (see text).

quences considered important for V–J rearrangement (Max et al., 1979; Sakano et al., 1979). Vγ5 has a stop codon (TGA) shortly after the initiation codon (residues 8–10, Figure 3). Vγ6 has a single base deletion (corresponding to residue 373, Figure 3), causing a frameshift and a further base change (residue 472; Figure 3), which results in an in-frame termination codon (residue 100, Figure 4). Vγ7 has a single base deletion (corresponding to residue 453, Figure 3), which produces a frameshift.

A Second Jγ Segment Upstream of Cγ1 Identified in a Rearranged Clone

DNA from the primary T-Prolymphocytic leukemia, designated F8 displayed only one rearranged allele when DNA was cleaved with HindIII, although two rearranged alleles were detected in BamHI-digested DNA (LeFranc and Rabbitts, 1985). Therefore, F8 cells must have one γ allele rearranged to Jγ1 and one rearranged to a region upstream of this segment. Analysis of λ phage clones isolated from a library of F8 DNA confirmed this conclusion. One allele was found to contain a rearrangement of the Vγ3 gene to Jγ1 (Figure 1C and described below). The other rearranged allele, present in λF1, has a restriction map identical with that of λSH2 up to the Vγ2 segment (Figure 5A); thereafter, the λF1 map corresponds to the unrearranged map of the region upstream from the Cγ1 gene present in λRγ (LeFranc and Rabbitts, 1985). Therefore, λF1 contains a rearrangement of Vγ2 to a segment 4.3 kb from Jγ1.

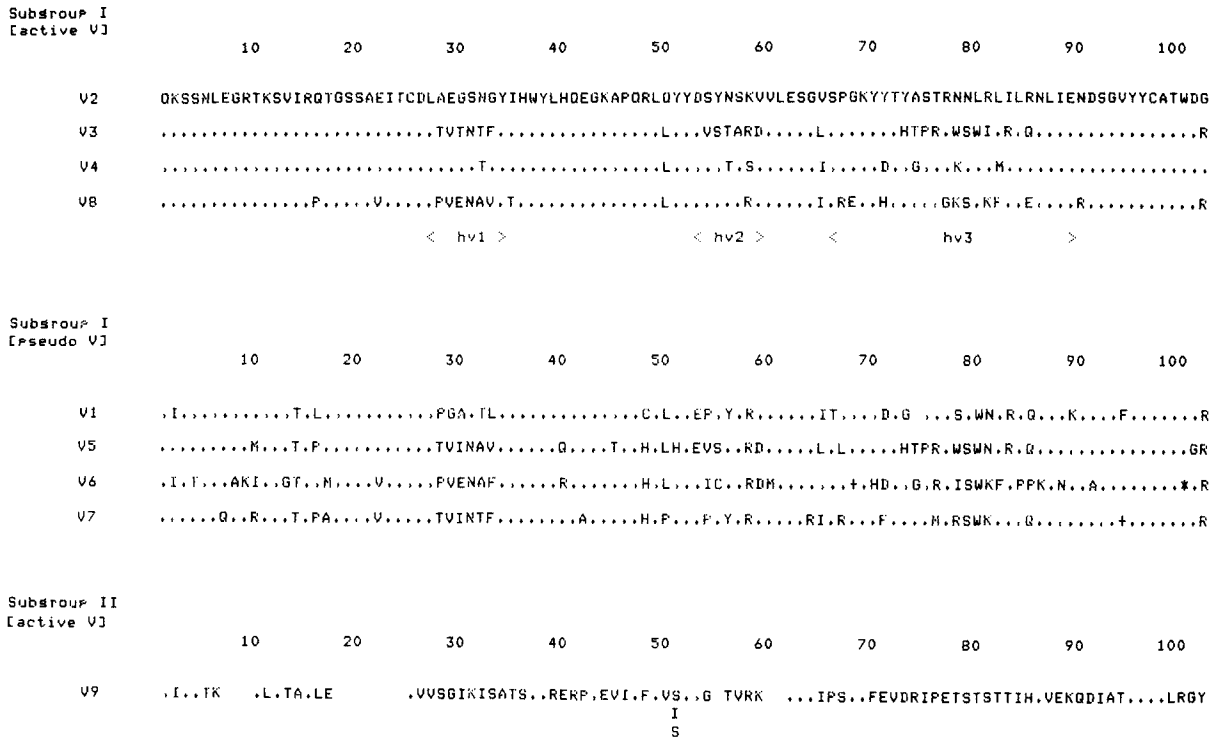


Figure 4. Derived Protein Sequences of Human Vγ Genes

The sequences shown in the single-letter code have been grouped into the four potentially active Vγl subgroup genes, the four pseudo-Vγl genes, and the single known Vγll gene. The protein sequences are all aligned to that derived from Vγ2; dots indicate identity and gaps of missing codons (this is most apparent in Vγ9, which is very different from Vγ2). hv: hypervariable region. Plus signs (+) in V6 and V7 represent frameshifts in these pseudogenes. The asterisk (*) in V6 represents an in-frame stop codon.

Nucleotide sequence analysis was carried out at the site of recombination in λF1 and compared with the analogous unrearranged DNA in λRγ and λSH2 (Figure 5B). These results showed that the Vγ2 segment had joined to a previously unidentified Jγ segment (here designated JγP). The V–J join is nonproductive, since the coding regions of Vγ2 and JγP are out of frame. The JγP segment has an RNA splice donor site (residue 92) at its 3′ end, but on the 5′ end there is very poor homology with the usual heptamer/nanomer sequences. It is interesting that JγP is 4 kb upstream of Jγ1. This is sufficient space for a further Cγ gene. If this is so, this gene must differ from the other Cγ genes, since they do not cross-hybridize (unpublished result).

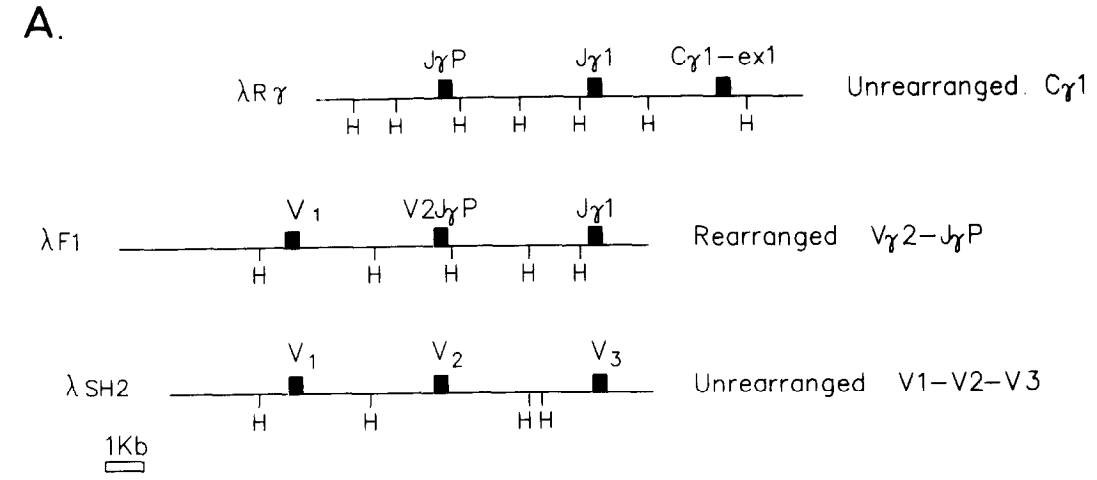
Rearrangement of the Active Vγ Genes in T Cells

Rearranged Vγ genes were isolated from F8, SUP-T1, K1010, and AT5B1. Each rearranged gene is illustrated in Figure 1C and listed in Table 1 (together with their surface phenotype), and each was fully sequenced in the Vγ–Jγ region. The sequences of three rearranged Vγ genes (Vγ2 from λF1 and Vγ3 from λF6 originating from F8; Vγ3 from λS12 originating from SUP-T1) were identical with the germ-line counterparts (except in the N-region, see below), so it appears that somatic mutation is as rare or nonexistent in human γ genes as in mouse (Hayday et al., 1985). For this reason and for clarity, only the junctional sequences of the rearranged Vγ genes are illustrated in Figure 6 and in Figure 5B. The germ-line Jγ1 and the 3′ end of Vγ3 are at the top of the figure for comparison. Productive (ie., in-frame) rearrangements occur in λF6 (which has the surface phenotype of a helper T cell), λS1, and λK20; λS13 and λA6 have nonproductive joins. Both Jγ1 and Jγ2 have productive joins associated with them, and a marked degree of N-region diversity (the seemingly random nucleotide alteration that can occur at V-D-J junctions [Alt and Baltimore, 1982]) is apparent (Figure 6). For example, the productive join in λF6 has resulted in the loss of the last two codons of the Vγ3 segment; on the other hand, the join in λS1 has altered the final two codons from Asp-Gly to Arg-Thr. There is no evidence for the involvement of Dγ segments in these rearranged genes, although the N-region diversity necessarily makes this assessment equivocal. It is, of course, possible that some of the putative N-region diversity arises from genetic polymorphism.

Table 2. Homology between Active Human Vγ Genes and Vγ2

Subgroup	Gene	% Homology	
		Nucleotide	Amino Acid
I	Vγ3	87	76
	Vγ4	95	91
	Vγ8	88	77
II	Vγ9	*	30

* No reasonable figure for nucleotide homology between subgroups I and II. Protein homology was calculated by introduction of gaps and is therefore an estimate.



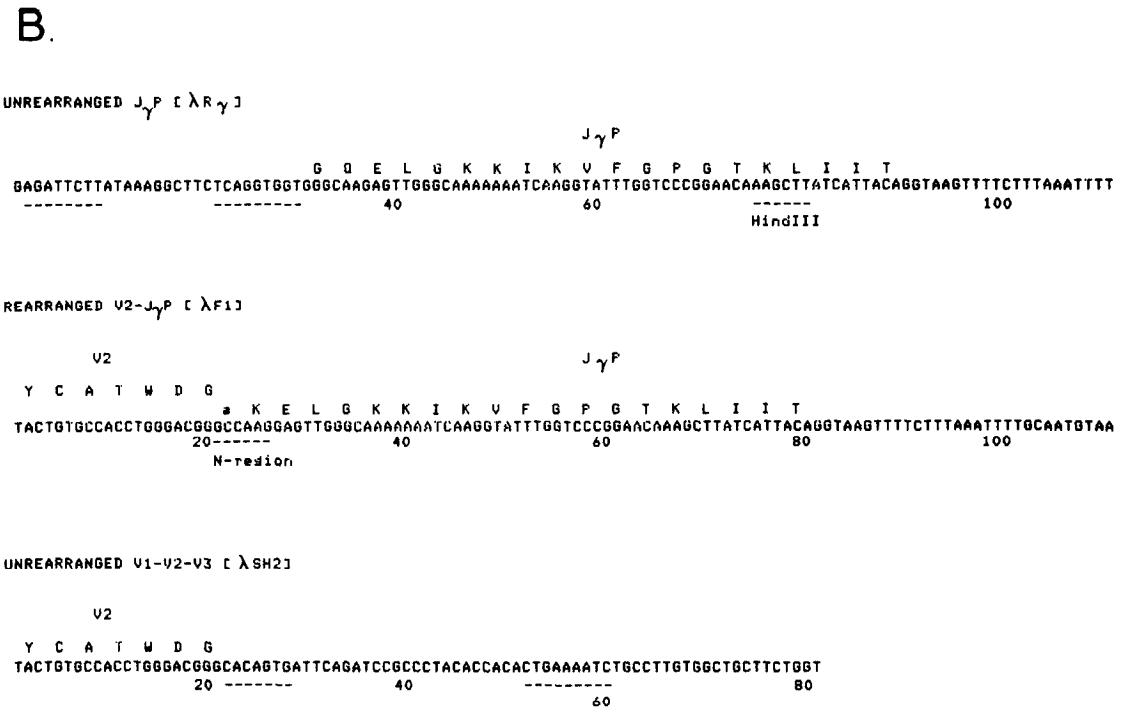


Figure 5. Vγ Rearrangement to a New Jγ Segment near Jγ1

(A) Restriction maps of λ clones containing unrearranged Jγ–Cγ genes from λRγ from Raji DNA (LeFranc and Rabbitts, 1985), or unrearranged Vγ1–Vγ2–Vγ3 genes from λSH2, and rearranged Vγ2 from λF1. H: HindIII.

(B) Nucleotide and derived protein sequences of the junction region from the unrearranged JγP in λRγ; of the rearranged Vγ2–JγP from λF1, and of the unrearranged Vγ2 in λSH2. The conserved heptamer and nanomer sequence adjacent to Jγ1 and Vγ2 in λRγ and λSH2 are underlined with dashes. The region of V–J joining (N-region) in λF1 is also underlined (the protein sequences are given in the single-letter code).

T Cell γ Gene Rearrangement in Human Thymus DNA

Based on hybridization and cloning data, the family of genes described here seems to represent a major portion of the human Vγ family. It should therefore be possible to detect a specific set of rearranged γ genes in human thymus DNA. Four thymus DNA preparations were made from 18 week or 20 week fetuses (in two cases, splenic DNA was also obtained). These DNAs were digested with HindIII and analyzed by Southern filter hybridization with the Jγ probe (shown in Figure 7C). In each thymus DNA sample, we found evidence for four distinct rearranged Jγ bands (Figure 7A) in addition to the germ-line fragments of 2.1 and 4.5 kb (the latter being a polymorphic band; eg., thymus sample TC in Figure 7A). No rearranged bands were found with the spleen DNA preparations, presumably because of the relatively low number of T cells in spleen. The presence of the unrearranged bands in the thymus DNA suggests that we are observing a subpopulation of cells undergoing γ rearrangement. Furthermore,

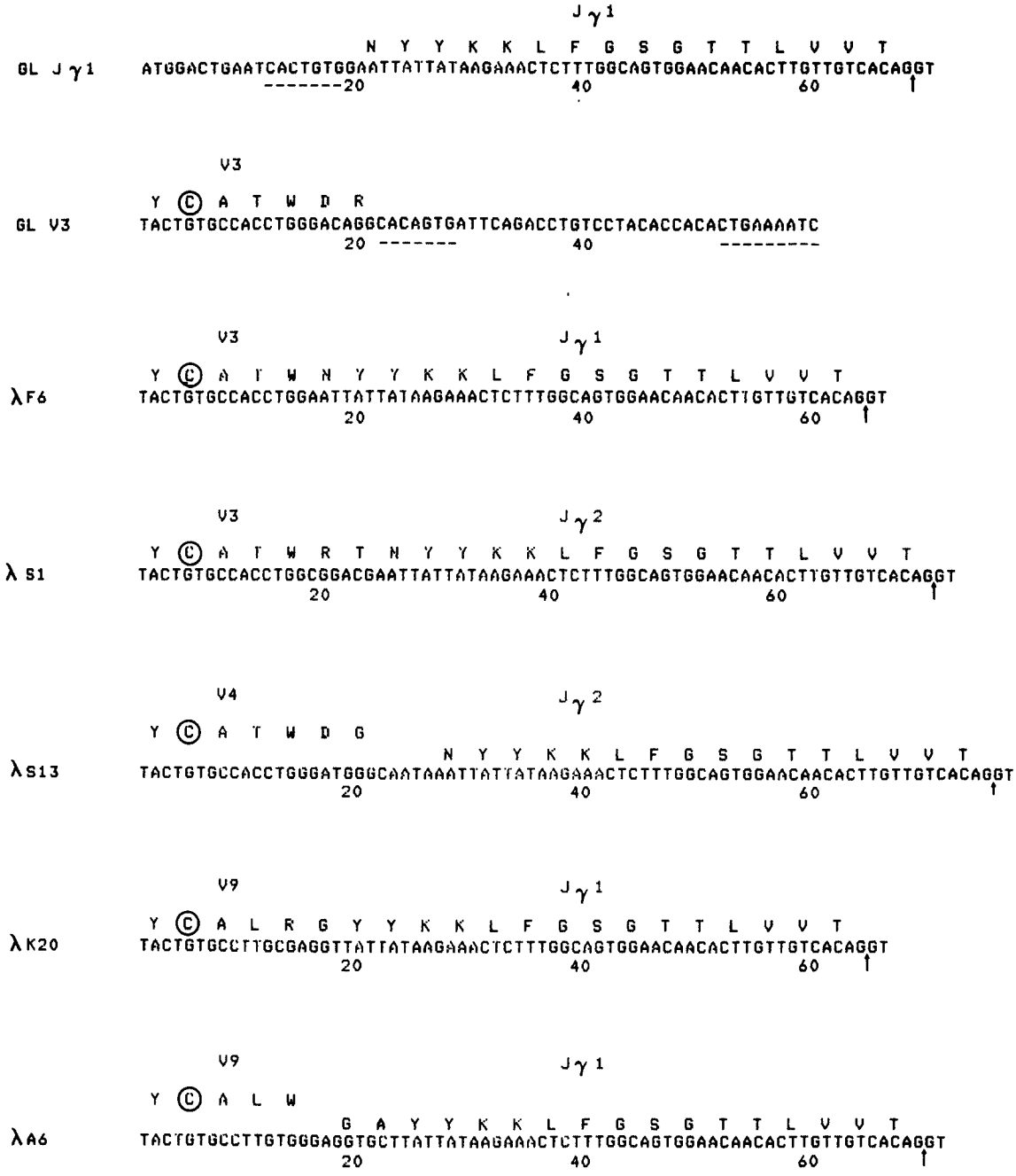


Figure 6. Junctional Sequences of the Various Vγ–Jγ Rearrangements Characterized from the T Cell Libraries

The sequences at the ends of unrearranged (GL) Jγ1 and Vγ3 are shown for comparison, with the conserved heptamer and nanomer sequences underlined. In each V gene the cysteine residues, probably involved in intrachain bridges, are circled, and RNA splice donor sites are marked with arrows. The nonproductively rearranged V genes are indicated by the displacement of the protein sequences of V and J segments. The full sequence of the Vγ gene from λA6 is not available at this time, so we cannot say whether it is exactly the same as that in λK20.

the specific but limited number of rearranged bands in thymus DNA indicates that a small number of Vγ genes rearrange in the thymus population. This conclusion is supported by the complexity of Vγl hybridization and the number of Vγ genes that we isolated. Alignment of the rearranged bands in the thymus samples with those in the four T cells used in this study (Figure 7A), shows that each rearranged band in the thymus has a counterpart in the T cell lines, except for the smallest rearranged thymus band (arrow in Figure 7A at about 2.5 kb). Thus the major rearrangements in the thymus cells seem to involve Vγ2, Vγ3, Vγ4, and Vγ9 (Vγ2 would comigrate with Vγ4 if rearranged to Jγ1 or Jγ2). We do not have a cloned example of a rearrangement involving Jγ8. However, the size of the smallest rearranged band in thymus is that expected if Vγ8 were joined to Jγ1 or Jγ2.

Thus it appears that rearrangement of each of the characterized active Vγ genes can be observed in whole

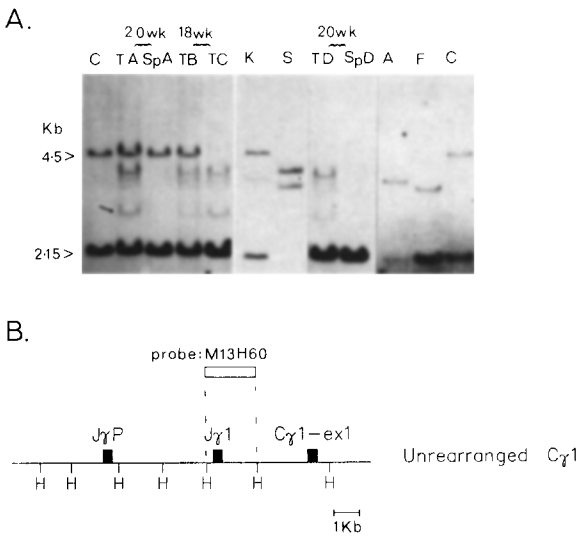


Figure 7. Genomic Hybridization Analysis of T Cell γ Rearrangement in Human Thymus

(A) Genomic DNA samples were completely digested with HindIII, fractionated in 0.8% agarose, and transferred to nitrocellulose. The filter was hybridized with the Jγ probe M13H60 (indicated in B) as described (Rabbitts et al., 1985). Exposure times were 2 weeks for the thymus and spleen DNA lanes and 2 days for the T cell lines or tumors. DNA samples are as follows: Colo320 (C), thymus (TA), and spleen (SpA) DNA from a 20 week fetus. TD, SpD are thymus and spleen DNA, respectively, from a 20 week fetus. TB and TC are separate 18 week thymus samples. K: K1010. S: SUP-T1. A: AT5B1. F: F8. The band marked by an arrow indicates a rearranged gene not represented in our phage clones, probably Vγ8.

(B) Restriction map of region from which the Jγ probe was derived.

thymus populations. The limited complexity of rearrangement within this population indicates that the five active Vγ genes identified are the major ones in the human γ system. In addition, DNA rearrangements observed in a panel of T cell lines and primary tumors can now be assigned to these five Vγ genes (LeFranc and Rabbitts, 1985). These conclusions, of course, are made assuming that the major rearrangements in the T cell populations occur to Jγ1 and Jγ2 and rarely to JP (as indicated from the structure of this JγP segment) or to any other as yet undefined Jγ segment. This seems likely, since we find only one allele out of sixty (ie., in F8) with rearrangement of JγP in T cell lines examined (unpublished data). We cannot be certain of the fate of the pseudo-Vγ genes in the thymus. However, the absence of rearrangements involving these segments in our cloned γ genes indicates that such rearrangements may be inhibited or selected against in the thymus population. A further unidentified rearranged band (4.6 kb) is faintly detected in some of the thymus DNA lanes. This may represent rearrangement of an unidentified Vγ gene.

Discussion

Germ-Line Diversity of Human T Cell γ Genes

The results described show that at least two distinct Vγ subgroups exist in human DNA. Members of the two Vγ subgroups are closely linked in genomic DNA since only 14.5 kb separates the Vγ8 subgroup I gene and the Vγ9 subgroup II gene (Figure 1A). At present, sequencing studies have defined only one member of subgroup II, but subgroup I contains at least eight genes. Hybridization studies with VγI and VγII probes suggest that the size of these families is unlikely to be much greater than the number of genes detected in our genomic clones. Sequence comparisons (Table 1) reveal considerable diversity among the four active VγI subgroup genes; the levels of amino acid identity between the products of these genes ranges from 72% (Vγ3 vs. Vγ8) to 91% (Vγ2 vs. Vγ4). (The VγII gene products show little identity with VγI gene products; only about 30% of amino acid residues are identical.) The distribution of sequence variation in the subgroup I genes is particularly striking when the derived protein sequences are aligned (Figure 4). It appears that, like the immunoglobulin genes, there are framework regions as well as fairly well defined hypervariable regions. This is indicative of the capacity to generate a variety of combining sites, and therefore of the potential ability to recognize a variety of antigens. This diversity within the active Vγ genes of subgroup I is far in excess of that present in the mouse Vγ genes described so far (Kranz et al., 1985). Although we have identified only two Vγ subgroups in man, the possibility that others exist must be considered.

Rearrangement of Human T Cell γ Genes

The rearrangements of human T cell γ genes are probably mediated by the conserved heptamer–nanomer sequences located at the 3′ side of the Vγ gene (spacing 23 bp) or upstream of the Jγ segments (spacing 12 bp). Studies of rearranged Vγ genes isolated from T cell tumors or lines showed that joining occurs predominantly with the Jγ1 or Jγ2 segments. One rearrangement involving an additional J segment (designated JγP) upstream of Jγ1 was detected. A productive rearrangement to JγP could produce another level of variability, since the sequence of JγP is markedly different from Jγ1 or Jγ2. The possibility of an analogous J segment upstream of Jγ2 has not been investigated, but there is space for it. It is also possible that another Cγ lies between JγP and Jγ1.

The DNA of SUP-T1 and F8 cells show both productive and nonproductive γ rearrangements (Figure 6 and Table 1). This provides evidence for allelic exclusion of the γ genes, as found in the immunoglobulin genes. Although K1010 has a productive rearrangement of VγII, AT5B1 does not. This means that AT5B1 could not have expressed the putative γ chain protein unless an unidentified Cγ gene was involved. This tumor may have arisen from a suppressor T cell because it has a $T8^+$, $T3^+$, $T4^-$ surface phenotype (Taylor and Butterworth, 1986). Since suppressor cells recognize antigen in the absence of MHC (Schwartz, 1985), lack of productive γ rearrangement in AT5B1 cells is indirect support for the idea that γ chain is required for MHC recognition in T cell differentiation (Heilig et al., 1985). The lack of productive γ rearrangement is also intriguing, because it suggests that human T cells can mature from the thymus in the absence of γ expression. In mouse, γ expression may not be re-

quired in helper T cells (Heilig et al., 1985). This may not be the situation in humans, since our cases include F8, which has $T4^+$, $T3^+$, $T8^-$ surface phenotype (characteristic of helper T cells), and a productive γ rearrangement (Table 1).

The Origin of Diversity in Human T Cell γ Genes

Our data show at least five potentially active Vγ genes and at least two Cγ genes. We have identified three Jγ segments, of which two (identical in their derived protein sequence) appear to be used in the majority of cases. Thus combinatorial diversity in human γ genes is restricted. A similar conclusion was reached in studies on the murine T cell γ genes (Kranz et al., 1985; Hayday et al., 1985).

Considerable variation in human Vγ genes is generated by N-region diversity resulting from V–J joining. N-region diversity is also important in the mouse system (Kranz et al., 1985). In the observed N-region diversity of the human genes, we find examples of both the addition and of the deletion of nucleotides at the V–J junctions (Figure 6). Our data provide no evidence for the existence of Dγ segments. We conclude that human γ gene diversity originates from diverse germ-line Vγ genes and V–J joining N-region diversity, and there is no evidence of somatic mutation.

We are still unable to say what the function of the putative T cell γ chain polypeptide is. The restricted rearrangement of the single Vγ gene in CTL and immature thymocytes of mouse has suggested a role of γ chain in the recognition of class I MHC molecules (Heilig et al., 1985). The possibility of a fourth type of gene that is involved in recognition of class II MHC products has been suggested (Heilig et al., 1985). In this respect, the existence of two distinct Vγ subgroups in man may be significant, since one of the two Vγ subgroup genes might well represent, not a fourth type of gene, but simply a different Vγ region capable of recognizing distinct antigen (eg, class II MHC). In any event, human helper T cells may productively rearrange and express γ chain, since we have one example of a cell with helper phenotype with a correctly joined Vγ segment. Clearly, the study of γ gene rearrangement in human T cell clones is important in the examination of questions relating to the function of the γ chain.

Experimental Procedures

Preparation and Analysis of λ Phage Libraries

Lambda phage libraries were prepared in BamHI-digested λ2001 vector (Karn et al., 1984). Genomic DNA was extracted from cells or from tumor material as previously described (Bentley and Rabbitts, 1981) and was partially digested with Sau3A. Fragments ranging from about 15–20 kb were selected after sucrose gradient fractionation, ligated to vector, and packaged by standard procedures. Recombinant libraries consisting of about 10^6 phage were screened with the respective nick translated probes in 6× SSC, 0.1% SDS, 50 μg/ml salmon DNA, 10 μg/ml E. coli DNA, 10× Denhardt's solution (Denhardt, 1966) at 65°C for 12–18 hr. Filters were washed in 6× SSC with 0.1% SDS at 65°C. Positively hybridizing phages were mapped by single and double digests of the enzymes indicated in the figures and by hybridizing with various probes also indicated by the legends.

Genomic DNA was prepared for genomic library preparation from the T cell primary tumor F8 (patient 8; Rabbitts et al., 1985), AT5B1 (Taylor et al., 1981), and from the established T cell lines SUP-T1 (Smith et al., 1984) and K1010 (A. Karpas, personal communication). A library was also made of DNA from an EBV-transformed B-lymphoblastoid cell line SH.

Filter Hybridization Analysis

Filter hybridization was carried out using nitrocellulose filters as described (Southern, 1975) with the hybridization conditions detailed for λ phage analysis. Filters were washed with either 6× SSC with 0.1% SDS, or 0.1× SSC with 0.1% SDS at 65°C. Probes were prepared by subcloning fragments from the λ phage clones into appropriately digested M13 or pUC vectors (Vieira and Messing, 1982).

DNA Sequencing Analysis

DNA sequencing was carried out by preparation of random sonicated clones in M13mp8 and sequence analysis using the dideoxy chain termination procedure (Sanger et al., 1980; Bankier and Barrell, 1983). Fragments to be subjected to these sequencing procedures were isolated from pUC or M13 clones by fractionating digests in low melting temperature agarose gels. The identified bands were sliced from the gel, the slice was melted at 65°C, and the DNA fragment was isolated using NACS columns. Nucleotide and protein alignments of sequence data was carried out using computer programs (Staden, 1986).

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